Lack of Genetic Differentiation in Aggressive and Secondary Bark Beetles (Coleoptera: Curculionidae, Scolytinae) from Arizona

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ABSTRACT Bark beetles (Coleoptera: Curculionidae, Scolytinae) play an important role as disturbance agents in ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) forests of Arizona. However, from 2001 to 2003, elevated bark beetle activity caused unprecedented levels of ponderosa pine mortality. A better understanding of the population structure of these species will facilitate analysis of their dispersal patterns and improve management strategies. Here, we use fluorescently labeled amplified fragment length polymorphism (fAFLP) analysis to resolve genetic variation among and within sampling locations in northcentral Arizona of *Ips pini* (Say), *Dendroctonus brevicomis* LeConte, and *D. frontalis* Zimmermann. We generated genetic fingerprints for >500 beetle specimens and analyzed genetic diversity. For all species, gene flow estimates among sampling locations were high, and significant population subdivision was not discernible across a large portion of ponderosa pine forests in Arizona. However, a weak relationship was detected with *I. pini* population structure and elevation. Because of the lack of genetic differentiation detected throughout the large study area, our findings suggest these insects are capable of long distance dispersal and exhibit a high degree of gene flow across a broad region. We conclude that our results are consistent with strong dispersal patterns and large population sizes of all three species.

KEY WORDS *Dendroctonus brevicomis*, *D. frontalis*, *Ips pini*, amplified fragment length polymorphism, genetic differentiation

In the western United States, most bark beetle (Coleoptera: Curculionidae, Scolytinae) species are native phloeophagous insects of forests that act as natural agents of disturbance that affect ecological function succession, nutrient cycling, and snag (i.e., dead tree) recruitment (Wood 1982, Stone and Wolfe 1996, Samman and Logan 2000, Kipfmueller et al. 2002). These insects use the phloem tissue just under the outer bark of Pinaceae to complete their life cycles (Wood 1982). Bark beetles can increase overall forest resilience and diversity through effects on patch dynamics and forest succession, thus creating habitat for a wide variety of organisms, and by providing an important food source for many species of insects and birds (Kolb et al. 1994, Samman and Logan 2000, Kipfmueller et al. 2002).

Although the diversity of bark beetles that inhabit ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) forests is quite high (at least 29 scolytines feed on ponderosa pine in Arizona; Wood 1982), only a few species can cause mortality of healthy trees. These are categorized as aggressive species (Furniss and Carolin 1977). These include some *Dendroctonus* species, which attack and kill trees through a pheromone-mediated mass attack (Miller and Keen 1960, Coulson 1979, Borden 1982, Cates and Alexander 1982, Wood 1982, Berryman et al. 1985, Samman and Logan 2000). Secondary invaders, including some *Ips* species, also must develop in dead trees, but are usually limited to attacking recently dead, weak, or dying trees (Furniss and Carolin 1977, Wood 1982, Raffa and Berryman 1983, Parker 1991, Kegley et al. 1997, Klepzig et al. 2001). It is important to note, however, that any aggressive species are capable of killing live trees (Coulson 1979, Borden 1982, Sturgeon and Mitton 1982, Raffa and Berryman 1983, 1987, Berryman et al. 1985).

In Arizona, recent outbreaks of *Ips pini* (Say) (pine engraver), *Dendroctonus brevicomis* LeConte (western pine beetle), and *D. frontalis* Zimmermann (southern pine beetle) have drastically altered the largest continuous ponderosa pine forest in the world. From 2001 to 2003, a combination of drought and existing high stand density facilitated increased bark beetle activity that has caused unprecedented levels of ponderosa pine mortality in central and northern Arizona. Aerial detection surveys estimated that millions of trees in this region were killed by bark beetles (USDA 2004, 2005). Because of this record high tree

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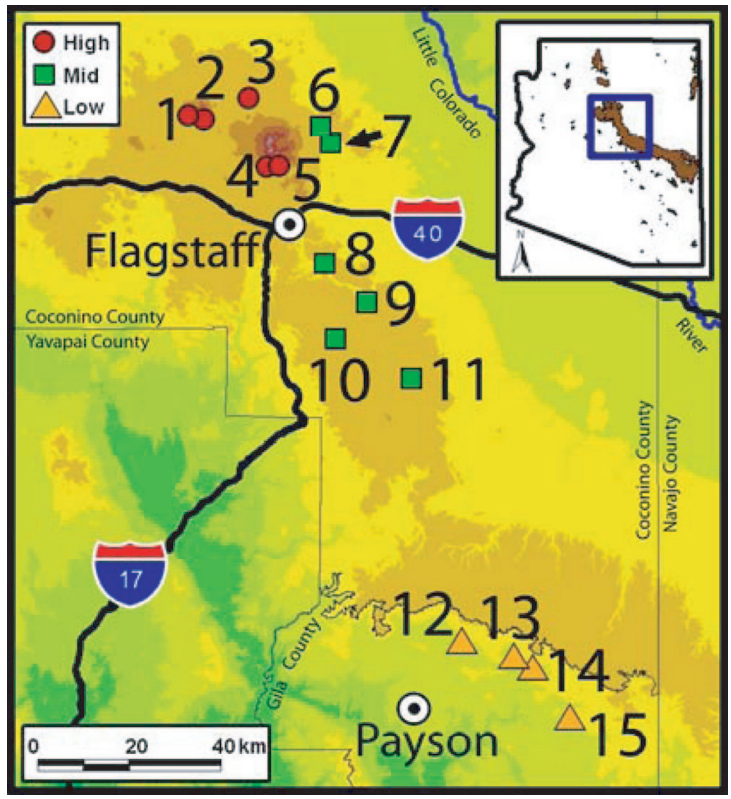

Fig. 1. Map of bark beetle sampling locations in northcentral Arizona. Unique identifiers for each site are indicated with numbers 1–15 (see Table 1). Green to dark brown color gradient corresponds to lower to higher elevations. Inset indicates expanded view location and approximate ponderosa type forest in brown.

mortality over such a large area, we hypothesized that bark beetles were dispersing rapidly across this landscape. To test this hypothesis, we used population structure estimates to elucidate the dispersal characteristics of these species. With the shift from pockets of pine mortality to large-scale mortality during these outbreak events, we postulated that dispersal estimates could help land managers decide how to manage these events in the future. If large scale dispersal was evident, localized techniques (e.g., application of aggregation pheromones) would be ineffective, and larger scale techniques (e.g., forest thinning) should be considered. However, if distinct population structure was detected, it may be more appropriate to focus on techniques for local scale protection. At the onset of our project, it was not known what regimen was present in these forested areas, and our main goal was to gain insight into this specific system.

Here, we use genetic tools to study the population structure of *I. pini*, *D. brevicomis*, and *D. frontalis*. The genetic technique used was fluorescently labeled amplified fragment length polymorphism (fAFLP) analysis, which uses randomly distributed polymorphic loci to generate high-resolution genetic fingerprints suitable for population level analyses (Travis et al. 1996, Keim et al. 1997, Garcia et al. 2002, Paupy et al. 2004). This approach has been used to successfully examine population structure in another species of bark beetle (Mock et al. 2007). Our specific goals were (1) to develop genetic markers that distinguish among and within sampling locations (i.e., populations) of *I. pini*, *D. brevicomis*, and *D. frontalis* and (2) to examine the population structure of these species to better understand their dispersal across a major portion of their range in northcentral Arizona.

Materials and Methods

Sample Collection. In 2004, adult specimens of *I. pini*, *D. brevicomis*, and *D. frontalis* were collected from 30 sampling locations in northcentral Arizona as part of a larger study examining bark beetle community structure across an elevation gradient (Williams et al. 2008). However, because of our efforts to control for multiple generations, only those collections spanning 36 consecutive days or less were used. This resulted in the use of specimens from only 15 of the 30 sampling locations (Fig. 1; Table 1). Collection of specimens occurred between 21 May and 25 June 2004. The elevation gradient followed one of three designations: high (≈2,500–2,700 m), mid (≈2,050–2,250 m), or low (≈1,600–1,750 m) elevation. At each site, two pheromone-baited Lindgren funnel traps were used for collection (Lindgren 1983). One trap

Table 1. Sampling locations for collecting selected *Dendroctonus* and *Ips* species attacking ponderosa pine in northcentral Arizona

Map ID no.[a]	Site name	Elevation category[b]	Elevation (m)	Sample size (collection date[c])		
				I. pini	*D. brevicomis*	*D. frontalis*
1	Newman Hill	High	2,532	20 (21 May)	18 (21 May)	23 (18 June)
2	Crowley Peak	High	2,507	23 (21 May)	—	—
3	Saddle Mountain	High	2,590	—	15 (28 May)	—
4	Freidlein Prairie	High	2,564	—	13 (28 May)	—
5	Orion Springs	High	2,627	—	—	14 (25 June)
6	Sunset Crater	Mid	2,155	23 (17 June)	18 (17 June)	—
7	Cinder Hills	Mid	2,146	22 (3 June)	21 (17 June)	24 (28 May)
8	Marshall Lake	Mid	2,195	—	23 (27 May)	15 (3 June)
9	Ashurst Lake	Mid	2,164	23 (17 June)	21 (3 June)	—
10	Rim	Mid	2,160	22 (9 June)	—	—
11	Cow Hill	Mid	2,208	—	21 (10 June)	21 (3 June)
12	Moore Creek	Low	1,735	23 (9 June)	—	21 (16 June)
13	Tonto Creek	Low	1,683	21 (2 June)	—	22 (16 June)
14	Christopher Creek	Low	1,734	22 (9 June)	—	—
15	Haigler Creek	Low	1,619	22 (9 June)	—	—
Total				221	150	140

[a] See Fig. 1.
[b] Elevation categories: high (≈2,500–2,700 m), mid (≈2,050–2,250 m), or low (≈1,600–1,750 m). See text for more details.
[c] All collections were made in 2004.

was baited for *D. brevicomis* using frontalin, *exo*-brevicomin, and myrcene, which also trapped *D. frontalis* (Williams et al. 2008). The other trap was baited for *I. pini* with a combination of lanierone and +03/−97 ipsdienol. Beetles were sorted and identified to species using morphological character examinations (Wood 1982). Whole beetles were stored at −20°C until DNA extraction, which was completed using a bead milling technique in conjunction with 96-well DNeasy Tissue Kits (Qiagen, Valencia, CA) as described by Allender et al. (2004). Final elution steps were modified to incorporate two washes of the DNA column with 60 μl of elution buffer such that the final volume contained genomic DNA in 120 μl.

fAFLP Procedure. DNA fingerprints were obtained for each beetle specimen using fAFLP analysis. We followed the general procedure of Vos et al. (1995) with the following modifications. Restriction-ligation (R-L) reactions were completed using 20 μl of DNA template, 1× RL buffer (50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 25 μg/ml BSA), and 5 U of both *Eco*RI and *Mse*I in a total volume of 50 μl. Samples were incubated for at least 1 h at 37°C before adding the additional 10 μl of ligation reagents (1× RL buffer, 0.12 mM ATP, 1 U T_4 DNA ligase, 1 μM *Eco* adapter mix, and 5 μM *Mse* adapter mix). Another 2-h incubation was performed at room temperature (between 20 and 25°C), followed by a 1:10 dilution of the R-L template in molecular grade water. Preselective polymerase chain reaction (PCR) was performed in 50-μl reaction volumes containing 1× PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 75 ng *Eco*RI primer with adenine as the selective nucleotide, 75 ng of *Mse*I primer with cytosine as the selective nucleotide, 1 U of *Taq* polymerase, and 5 μl of R-L template. The cycling parameters started with an initial 2 min at 72°C, followed by 25 cycles of 20 s at 94°C, 30 s at 56°C, and 2 min at 72°C. The final step was a 30-min elongation step at 60°C. Samples were diluted 1:10 in molecular grade water.

The second selective PCR used 5′ fluorescently labeled *Eco* primers and unlabeled *Mse* primers. The following four primer combinations were used on all samples: 6FAM-*Eco*AAC-*Mse*CCA, 6FAM-*Eco*AAC-*Mse*CGA, HEX-*Eco*ACG-*Mse*CCA, and HEX-*Eco*ACG-*Mse*CCA. Using 3 μl of diluted products from above, the second selective PCR was performed in 20-μl reaction volumes containing 1× PCR buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs, 2% of volume HiDi Formamide (Applied Biosysyems, Foster City, CA), 1 U *Taq* polymerase, 0.05 μM fluorescently labeled selective *Eco*RI primer, and 0.25 μM selective *Mse*I primer. The PCR cycling parameters described in Travis et al. (1996) were used, except the final cycle was repeated an additional 20 times for a total of 30 cycles. Capillary gel electrophoresis and data analysis was completed using a 3730xl DNA sequencer (Applied Biosystems) and Genemapper 3.7 software (Applied Biosystems).

Genetic Analyses. Genetic variation was evaluated using nonmetric multidimensional scaling (NMDS) ordination. This method is similar to principle components analysis, by providing a visual representation, but makes fewer assumptions about the type of data and relationships among the samples while still conserving rank distance relationships in low-dimensional ordination space (Clarke and Warwick 2001). In addition, by applying analysis of similarity (ANOSIM; Clarke 1993), an analog of analysis of variance (ANOVA), statistical inference can be applied to the similarity distance rankings created by the ordination (Clarke and Warwick 2001). The NMDS ordinations and ANOSIM analyses were completed using the software program DECODA (Minchin 1991). ANOSIM analyses used 1,000 permutations to generate $R\{R = (\text{mean ranks between groups} - \text{mean ranks within groups}) / [N(N-1)/4]\}$ and P values. Bonferroni cor-

rections were applied to subsequent post hoc analyses based on a global α level of 0.05.

An estimate for overall mean pairwise genetic distance was used to compare the relative amount of genetic diversity within each species. These distance estimates were compared with one another to determine the overall genetic diversity of each species. Genetic distances were computed using MEGA 3.0 software (Kumar et al. 2004).

We used analysis of molecular variance (AMOVA) and NMDS ordination to assess population structure patterns within each species. This combination allowed us to examine gene flow patterns (AMOVA) and resolve possible sampling location differences (NMDS ordination). AMOVA uses Φ, an analog of F statistics (Weir and Cockerham 1984), to partition the total variance into hierarchical levels of subdivision (Excoffier et al. 1992). We used the software package Genetic Analysis in Excel (GenAlEx) version 6 (Peakall and Smouse 2006) to partition genetic variation among elevations, among sites within elevations, and within sites using pairwise distance estimates. NMDS ordinations and ANOSIM analyses were used to examine whether beetles collected from a given sampling location, or from the same elevation band, formed distinct genetic groups. When applicable (i.e., significant global ANOSIM test), post hoc pairwise comparisons were used to examine differences among specific populations.

Results

Every beetle we examined had a unique fAFLP signature and exhibited an average of 30 markers present per individual. Using the four primer combinations, a total of 333 markers were detected with 255 polymorphic among our samples. Of these 255 polymorphic markers, 161, 138, and 133 were polymorphic within *I. pini*, *D. brevicomis*, and *D. frontalis*, respectively. The number of polymorphic markers yielded by each primer combination was 55, 58, 66, and 76. Also, an overall error estimate of 2.6% for fAFLP marker reproducibility was determined using a sample of nine replicates from each species across the four primer combinations.

The results for assessing genetic variation among species using NMDS ordination (Fig. 2) indicated separation of the three species into three separate clusters. It was further supported by the ANOSIM test ($R = 0.9998$, $P < 0.001$). In addition, the pairwise comparisons showed that each of the three clusters present in the NMDS ordination (Fig. 2) were statistically different from the others. These results were not surprising but are included here to show the power of the fAFLP approach for distinguishing among species, as well as within species.

The mean genetic distance estimates for all three species were low, indicating that each exhibited similar banding patterns among individuals, which suggests homogenous populations. The result for *I. pini* was the highest (0.104), indicating that it contained the greatest amount of diversity of the three species.

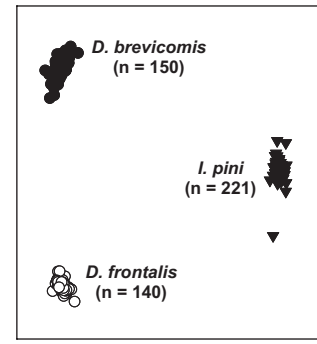

Fig. 2. NMDS ordination plot of three bark beetle species using 255 polymorphic AFLP markers.

It was followed by *D. brevicomis* with a value of 0.093 and then *D. frontalis* with a value of 0.086.

The AMOVA and NMDS ordination results for *I. pini* indicated very little population structure. AMOVA (Table 2) results determined that variation among elevations, among sites within elevations, and within sites was 1.4, 2.4, and 96.2%, respectively. The NMDS ordinations and ANOSIM analyses nevertheless showed significant differences among both sampling locations ($R = 0.0936$, $P < 0.001$; Fig. 3A) and elevations ($R = 0.0458$, $P = 0.003$). Post hoc analysis of sampling locations (Table 3) indicated that some of the sites significantly differed from one another but without clear patterns. In addition, post hoc analysis of elevations showed that beetles from low elevation sites formed a distinct group that was significantly different from beetles from both mid and high elevation sites.

Population structure for *D. brevicomis* was shown by AMOVA and NMDS ordination results. The AMOVA (Table 2) results indicated that variation among elevations, among sites within elevations, and within sites was 0, 5.2, and 94.8%, respectively. The NMDS ordinations for sampling locations (Fig. 3B) and elevations indicated high diversity among samples but no clear groupings. ANOSIM results indicated significant differences among sampling locations ($R =$

Table 2. AMOVA results for *I. pini*, *D. brevicomis*, and *D. frontalis* collected in ponderosa pine forests of northcentral Arizona

Source	df	SS	% variation	*P*
I. pini				
Among elevations	2	64.9	1.4	<0.01
Among sites, within elevations	7	138.3	2.4	<0.01
Within sites	211	2,677.1	96.2	<0.01
D. brevicomis				
Among elevations	1	19.5	0	0.790
Among sites, within elevations	6	137.7	5.2	<0.01
Within sites	142	1,592.5	94.8	<0.01
D. frontalis				
Among elevations	2	45.5	0	1.000
Among sites, within elevations	4	115.4	8.5	<0.01
Within sites	133	1,359.0	91.5	<0.01

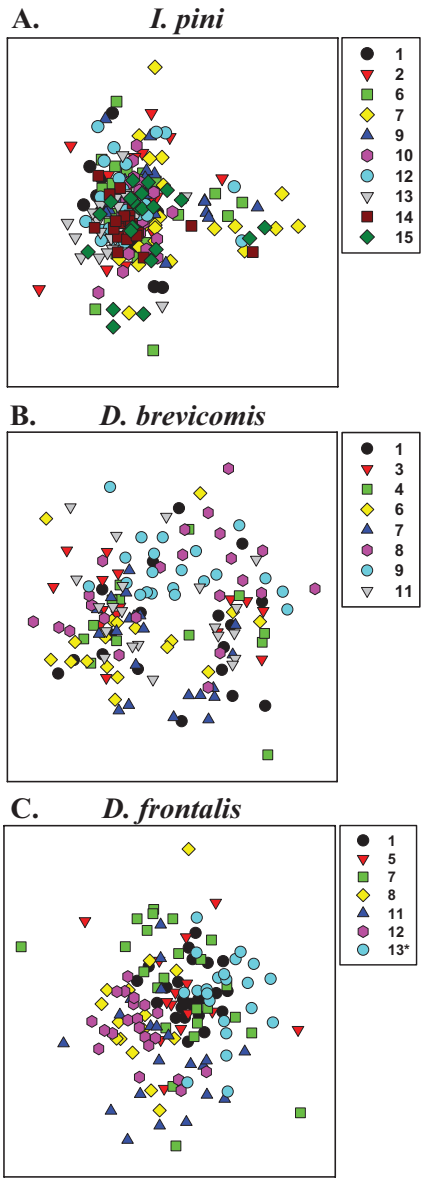

Fig. 3. NMDS ordination plots of sampling locations for *I. pini* (A), *D. brevicomis* (B), and *D. frontalis* (C) collected in northcentral Arizona. Numbers refer to specific sampling locations (see Fig. 1 and Table 1). *Sites that significantly differed from all others using ANOSIM pairwise estimates (see Table 3).

0.1390, $P < 0.001$) but no differences among elevations ($R = 0.0337$, $P = 0.121$). Subsequent post hoc analysis of sites (Table 3) showed that site 9 differed from all the other sites except site 3.

Population structure for *D. frontalis* was also evident from AMOVA and NMDS ordination results. Variation among elevations, among sites within elevations, and within sites was 0, 8.5, and 91.5%, respectively (Table 2). The NMDS ordination for sampling locations (Fig. 3C) and elevation indicated some sample diversity with population subdivision evident. As with *D. brevicomis*, the overall ANOSIM tests detected significant differences only among sampling locations ($R = 0.2526$, $P < 0.001$) and not among elevations ($R = 0.0044$, $P = 0.387$). Further post hoc analysis of sites (Table 3) showed that only site 13 was significantly different from all other locations.

Discussion

Among Species Genetic Variation. We conclude that fAFLP analysis provides powerful markers for detecting genetic variation among the three examined bark beetle species. The high level of differentiation observed in the overall NMDS ordination (Fig. 2) and subsequent ANOSIM results suggest these species have very different genetic signatures, as expected for distinct species (Wood 1982).

Because each beetle was assigned to species before genetic analysis, we were able to test the reliability of routine morphological classification versus genetic evaluation. We found that only 11 of the 511 beetles that we examined were inconsistent between morphology and the NMDS ordination. After checking the identities of these individuals, we found they were from one collection site and collected on the same date; these discrepancies were only detected between the two *Dendroctonus* species. To further evaluate these beetles for species determination, we sequenced a portion of their cytochrome oxidase I gene. These sequences were identical to sequences generated in previous studies (Kelley and Farrell 1998, Cognato and Sperling 2000) and therefore consistent with the fAFLP data (C.J.A., unpublished data). These results suggest that the 11 individuals were incorrectly labeled by morphology and that the overall error rate of the morphological classification method was 2% in this study.

Genetic Variation Within Species. With respect to genetic variation within the three species, we conclude that there was an overall lack of genetic differentiation among sampling locations and, therefore, that most sites were part of a larger regional (e.g., northcentral Arizona) population. The overall mean genetic distance results were suggestive of little differentiation, with estimates that were low and similar for all three species. The AMOVA results (Table 2) indicate the presence of high gene flow and a lack of genetic differentiation among sites for all species. Also, there was difficulty in discerning clear population subdivision patterns using NMDS ordination (Fig. 3) and ANOSIM analyses (Table 3), indicating a lack of separation into groups with respect to site and elevation differences. These attributes may be a result of dispersal capabilities, previous bottleneck events that lowered their genetic diversity, or selection pressures influencing their population diversity.

We attribute the lack of differentiation among sampling locations to the moderate to strong dispersal capabilities of these species (Cognato et al. 1999). Because our results indicate high gene flow and limited population structure among our sampling loca-

Table 3. Mean pairwise genetic distances between sampling locations of *I. pini*, *D. brevicomis*, and *D. frontalis* collected in ponderosa pine forests of northcentral Arizona

Elevation category	Map ID no.[a]	Map ID no.[a] and elevation category													
		High							Mid				Low		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
I. pini site comparisons															
High	2	0.097		—	—	—			—			—			
	6	0.109	0.113	—	—	—			—			—			
Mid	7	0.110	0.113[b]	—	—	—	0.117		—			—			
	9	0.107	0.111	—	—	—	0.124	0.118	—			—			
	10	0.089	0.096	—	—	—	0.107	0.107[b]	—	0.105		—			
Low	12	0.106[b]	0.110[b]	—	—	—	0.120	0.121[b]	—	0.117	0.106[b]	—			
	13	0.093[b]	0.098[b]	—	—	—	0.101[b]	0.111[b]	—	0.108[b]	0.092[b]	—	0.099		
	14	0.104[b]	0.108[b]	—	—	—	0.064	0.115[b]	—	0.120[b]	0.101[b]	—	0.114	0.095[b]	
	15	0.106[b]	0.110	—	—	—	0.065	0.114	—	0.120	0.102	—	0.116	0.097[b]	0.059
D. brevicomis site comparisons															
High	3	0.090	—			—					—	—	—	—	—
	4	0.094	—	0.092		—					—	—	—	—	—
Mid	6	0.102	—	0.099	0.103	—					—	—	—	—	—
	7	0.096	—	0.096	0.098	—	0.100				—	—	—	—	—
	8	0.092	—	0.091	0.095	—	0.102	0.097			—	—	—	—	—
	9	0.088[b]	—	0.085	0.091[b]	—	0.101[b]	0.096[b]	0.087[b]		—	—	—	—	—
	11	0.087	—	0.086	0.091	—	0.096	0.092	0.087	0.086[b]	—	—	—	—	—
D. frontalis site comparisons															
High	5	0.083	—	—	—		—			—	—			—	—
Mid	7	0.088[b]	—	—	—	0.093	—			—	—			—	—
	8	0.081[b]	—	—	—	0.083	—	0.088[b]		—	—			—	—
	11	0.089[b]	—	—	—	0.090	—	0.098[b]	0.079	—	—			—	—
Low	12	0.078[b]	—	—	—	0.080[b]	—	0.085[b]	0.063	—	—	0.075[b]		—	—
	13	0.086[b]	—	—	—	0.094[b]	—	0.100[b]	0.095[b]	—	—	0.097[b]	0.093[b]	—	—

[a] See Fig. 1 and Table 1.
[b] Significant differences between populations using ANOSIM pairwise population comparisons with Bonferonni correction (global $\alpha < 0.05$).

tions, we postulate that the scale of our study area was well within the dispersal capabilities of the three bark beetle species, with range-wide dispersal occurring over a single or perhaps a few generations. Our results are consistent with previous work showing that strongly dispersing phytophagous insects exhibit homogenizing effects because of gene flow over hundreds of kilometers (Peterson and Denno 1998). The greatest distance within this study area, between the most northern and southern sites, was ≈120 km (Fig. 1).

Although our results indicated similar population structure for all three species, there were some differences. As previously mentioned, *I. pini* exhibited the greatest amount of differentiation (e.g., overall mean genetic distance of 0.104), but this did not contribute to a higher degree of variation detected among sites compared with the two *Dendroctonus* spp. Also, the NMDS ordination for *I. pini* (Fig. 3A) detected two clusters of sampling locations that did not show a relationship to geographic position. These results suggested that population subdivision may be present, which is consistent with a previous study that observed two distinct genetic lineages of *I. pini* in Arizona (Cognato et al. 1999). In contrast, the two *Dendroctonus* spp. contained lower overall mean genetic distance estimates, an apparent indication of less differentiation and higher gene flow than *I. pini*, but this was not the case for both species. Specifically, *D. brevicomis* showed the weakest population differentiation patterns, which was further supported by few significant differences detected among sites with the pairwise comparisons (Table 3). We believe this was because of the close proximity of the sampling locations because there was less geographic distance, ≈70 km, between the most northern (site 3) and southern (site 11) collection sites for this species (Fig. 1). However, *D. frontalis* exhibited the most variation among sites, although this is not immediately apparent by the NMDS ordination (Fig. 3C). This variation becomes most apparent from the pairwise comparisons (Table 3), which detected significant differences between many of the sampling locations. This differentiation was present even though the mean genetic distance was the lowest (0.086) of the three species. Site 13 was not only found to be significantly different from all other sampling locations (Table 3), but it was also the most geographically isolated.

Because of the sampling locations used in this study, we were also able to test for the influence of elevation with respect to gene flow within each species. Elevation has been thought to play an important role in limiting bark beetle species distributions (Wood 1982, Lombardero et al. 2000). However, our results indicate that elevation variation within suitable habitat may not be an important factor, because only the results for *I. pini* suggested an effect of elevation on gene flow. AMOVA results (Table 2) showed that a

weak (1.4% of variation) but significant ($P < 0.01$) proportion of genetic variance within this species was linked to the three elevation designations. In addition, the ANOSIM testing for elevation only indicated significant differences among elevations for *I. pini*, which agreed with the AMOVA results. It is not clear why elevation differences may serve as a minor dispersal barrier for *I. pini*.

Implications for Bark Beetle Management and Future Experimentation. The lack of genetic differentiation detected among sampling sites within each species is consistent with large dispersal distances and large population size, at the time of this study and in the past. This indicates that regional management is needed because our results imply that outbreaks of *I. pini*, *D. brevicomis*, and *D. frontalis* will have an impact over the entire region, not just in the areas where ponderosa pine mortality is currently high. It also suggests that the recent outbreaks may not have increased the population sizes significantly because there may have always been a large beetle presence in the forest. Because our sampling locations span over such a large portion of the continuous ponderosa pine forests of Arizona, we speculate that our results are most likely universal throughout the whole region (Fig. 1).

Acknowledgments

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